REMARKS

Applicant wishes to thank Examiner Turner and Supervisor Kunz for the courtesy extended to the inventor, Dr. Wang, and the representative, Nancy Vensko, attorney of record, on May 20, 2004. The Interview Summary Form PTOL-413 summarizes the discussions held at the personal interview. The present response to the outstanding Office Action includes the substance of the Examiner Interview.

A. Disposition of Application

Claims 29-38 are pending in the application. Claims 1-28 have been canceled as being drawn to non-elected subject matter (or, for Claim 23, as being drawn inter alia to a non-elected species). Claim 23 has otherwise been canceled and Claims 29-38 have been added to better describe the claimed invention as binding vs. signal transduction with binding being the first step. Thus the amendment is made for reasons unrelated to patentability. Support for the amendment is found throughout the Specification, for example, as discussed below. The Brief Description of the Drawings has been amended to reflect the proper views, and the Specification has been amended to conform thereto. The Specification has also been amended to correct minor editorial problems. Under MPEP 608.01, the Specification has additionally been amended to delete the embedded hyperlink. The Title has been amended to reflect the elected subject matter. No new matter is being added herewith.

B. The Interaction of FRPL1 with Serum Amyloid A (SAA)

According to the illustration, attached for the convenience of the Examiner, the claims are related to the interaction of FRPL-1 with serum amyloid A (SAA). Previous investigators have reported that the acute phase protein serum amyloid A (SAA) is a potent chemoattractant for human leukocytes in vitro and mouse phagocytes in vivo. To identify the signaling mechanisms, the present investigators evaluated patterns of cross-desensitization between SAA and other leukocyte chemoattractants. They found that the chemotactic bacterial peptide, *N*-formyl-methionyl-leucyl-phenylalanine (fMLP), was able to specifically attenuate Ca^{2+} mobilization in human phagocytes induced by SAA, but only at very high concentrations, suggesting that SAA uses a low affinity fMLP receptor. Next they demonstrated that SAA selectively induced Ca^{2+} mobilization and migration of HEK cells expressing FPRL1, a human seven-transmembrane

domain phagocyte receptor with low affinity for fMLP, and high affinity for lipoxin A4. Furthermore, radiolabeled SAA specifically bound to human phagocytes and FPRL1-transfected 293 cells. In contrast, SAA was not a ligand or agonist for FPR, the high affinity fMLP receptor. Thus, SAA is the first chemotactic ligand identified for FPRL1.

Assays of Ca^{2+} mobilization have provided a useful approach to identify ligands for chemoattractant receptors. In primary cells, cross-desensitization of Ca^{2+} transients is often due to two agonists acting at the same receptor. Since SAA induced Ca^{2+} mobilization in phagocytes, the investigators used cross-desensitization to characterize the molecular nature of SAA receptor(s). In a series of cross-desensitization experiments, SAA at 1 μM did not desensitize the Ca^{2+} flux in monocytes or neutrophils induced by chemokines such as monocyte chemotactic protein (MCP)-1, RANTES, MCP-3, macrophage inflammatory protein (MIP)-1α, IL-8, and stromal cell-derived factor (SDF)-1α. Therefore, SAA is unlikely to share a receptor with any of the chemokines tested. SAA also did not attenuate the cell response to the bacterial chemotactic N-formylated peptide fMLP when fMLP was used at 100 nM (10^{-7} M) (Spec. at Fig. 1 A). However, in reciprocal tests, fMLP at 100 nM showed a partial desensitizing effect on SAA-induced Ca^{2+} mobilization in monocytes (Spec. at Fig. 1 B). Furthermore, the cell response to SAA was completely desensitized by higher concentrations of fMLP (10^{-3} M = 1 mM, Spec. at Fig. 1 C), suggesting that SAA might use a receptor(s) for which fMLP has low affinity.

Since fMLP is known to induce Ca^{2+} mobilization in phagocytes through at least two seven-transmembrane, G protein-coupled receptors, FPR and FPRL1, the investigators tested the effect of SAA using cells transfected to express these receptors that originally were not responsive to fMLP stimulation. fMLP in a wide range of concentrations induced Ca^{2+} mobilization in FPR-transfected rat basophil leukemia cell line (ETFR cells), with an EC_{50} of 10 pM. In contrast, the EC_{50} for fMLP to induce Ca^{2+} mobilization in FPRL1 transfected cells (FPRL1/293 cells) was much higher at 10 μM (Spec. at Fig. 2 A). These results confirmed the previous observation that FPR is a high affinity receptor for fMLP, whereas FPRL1 has a much lower affinity. rhSAA induced Ca^{2+} mobilization in cells transfected with FPRL1 (FPRL1/293 cells (Spec. at Fig. 2 B), but not in FPR-expressing cells or mock-transfected 293 cells (Spec. at Fig. 2, C and D). The EC_{50} of rhSAA on FPRL1 transfected cells was 250 nM, suggesting that SAA activates FPRL1 with higher efficacy than fMLP. This was supported by studies of cross-desensitization of Ca^{2+} flux between SAA and fMLP in FPRL1/293 cells. As shown in Spec. at

Fig. 2 E and F, although sequential stimulation of FPRL1/293 cells with SAA and fMLP resulted in bidirectional desensitization, SAA was able to desensitize the cell response to a 100-fold excess of fMLP. In contrast, fMLP at 100-fold excess of SAA only partially desensitized the effect of SAA.

Leukocyte infiltration in vivo is considered to be based on migration of cells toward a gradient of locally produced chemoattractant(s). This process can be emulated by in vitro assays of chemotaxis, which provides a very sensitive and biologically relevant means of evaluating the function of cloned chemoattractant receptors. Since SAA has been shown in previous studies to induce leukocyte infiltration in vivo and chemotaxis in vitro, the investigators next investigated whether SAA could induce cell migration via FPRL1. FPRL1/293 cells showed a potent migratory response to SAA with an EC_{50} of 200 nM, but these cells failed to migrate in response to a wide range of concentrations of fMLP (Spec. at Fig. 3 A). In contrast, fMLP induced migration of ETFR cells at nanomolar range concentrations, whereas the same cells did not migrate in response to SAA (Spec. at Fig. 3 B). The chemotaxis experiments indicate that fMLP is only a partial agonist for FPRL1 since it did not induce cell migration through FPRL1. On the other hand, SAA showed full agonist activity on FPRL1. Both SAA-induced Ca^{2+} mobilization and chemotaxis in FPRL1/293 cells were inhibited by pretreatment of the cells with pertussis toxin but not cholera toxin in correlation with the observation in native cells, suggesting activation of G protein of the Gi type is required for SAA signaling through FPRL1. In addition, since SAA can form complexes with HDL, which acts as a natural inhibitor of SAA, the investigators examined the effect of HDL on the chemotactic activity of SAA for FPRL1/293 cells. Spec. at Fig. 3 C shows that HDL, whether preincubated with SAA or simultaneously added to SAA, completely abolished SAA-induced FPRL1/293 cell migration. In contrast, the same concentration of HDL did not affect migration of FPR-expressing ETFR cells induced by fMLP. These results confirmed that HDL specifically inhibited the agonist activity of SAA on FPRL1.

To further verify the usage of FPRL1 by SAA, the investigators performed ligand binding experiments. Spec. at Fig. 4 shows that radio-iodinated SAA specifically bound to FPRL1/293 cells with an estimated K_d at 64 nM and 42,000 binding sites per cell (Spec. at Fig. 4 A). ^{125}I-labeled SAA also specifically bound to monocytes (Spec. at Fig. 4 B) and neutrophils (K_d = 45 nM, R = 6,700/cell) with K_d values comparable to those achieved with FPRL1/293 cells. In the displacement assay, unlabeled SAA in a dose-dependent manner inhibited its own binding to

monocytes (Spec. at Fig. 4 C), neutrophils and FPRL1/293 (Spec. at Fig. 4 D) with an IC_{50} at ~50 nM. In contrast, unlabeled fMLP at high concentrations (≥10 μM) only partially competed with ^{125}I-SAA for binding. These results confirm SAA to be a far more efficient agonist for FPRL1 than fMLP.

In this Specification, the investigators demonstrate that SAA uses FPRL1, a seven-transmembrane, G protein-coupled receptor expressed on phagocytes as a chemotactic receptor, suggesting a molecular basis for our previous observations that SAA is a potent chemoattractant and activator for human peripheral blood monocytes and neutrophils. In addition to SAA, FPRL1 has previously been shown to be a low affinity receptor for fMLP and a high affinity receptor for lipid metabolite LXA4 and its analogues. The present data suggest that fMLP is a partial agonist incapable of inducing chemotaxis via FPRL1 in this model system. Analysis of LXA4 induction of chemotaxis via FPRL1 has not been reported. Thus, SAA is the first chemotactic agonist identified for FPRL1.

C. Support for Amendment

The amendment is supported throughout the specification, for example, as indicated below in the Support Chart.

Support Chart

Claim	Support in Specification
29	7:18-30; 49:23-51:9; and 74:28-75:13 (and 73:17-18 for the explanation of the abbreviation FPRL1)
30	7:28
31	7:28
32	7:29
33	19:26-45:11
34	11:19-29
35	9:22-10:6
36	11:19-29
37	11:10-18
38	67:13-17

D **Compliance with 35 USC 101, 112/1, and 112/2**

The Patent Office rejected Claim 23 under 35 USC 101, 35 USC 112/1, and 35 USC 112/2 as lacking utility, "how to use" enablement, and indefiniteness. The claims must meet these requirements all related to utility for screening methods. The added claims have been conformed to the claims for screening methods adjudged to be valid in USP 5,837,479 by Univ. of Rochester v. G.D. Searle & Co., 69 USPQ2d 1886 (Fed. Cir. 2004), thus the claims fully comply with 35 USC 101, 112/1, and 112/2 as meeting the requirements for utility, "how to use" enablement, and indefiniteness.

E. **Compliance with Written Description Requirement**

The Patent Office rejected Claim 23 under 35 USC 112/1 as failing to meet the written description requirement. According to MPEP 2163, "[t]he written description requirement for a claimed invention may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus." The phrase "or conservative variants or functional fragments thereof" is at issue. The phrase has been replaced with the phrase "carboxy truncation, amino truncation, or internal truncation of SAA having a sequence selected from the group consisting of SEQ ID NOs: 2-301." Support is 19:26-45:11. Here, the written description requirement is met by disclosure of relevant identifying characteristics, i.e., by a combination of such identifying characteristics (structure) sufficient to show the applicant was in possession of the claimed genus.

CONCLUSION

In view of the above, it is submitted that the claims are in condition for allowance. Reconsideration and withdrawal of all outstanding rejections are respectfully requested. Allowance of the claims at an early date is solicited. If any points remain that can be resolved by telephone, the Examiner is invited to contact the undersigned at the below-given telephone number.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: 6/25/04

By: _____

Nancy W. Vensko
Registration No. 36,298
Attorney of Record
Customer No. 20,995
(805) 547-5585

AMEND
O:\DOCS\NWV\NWV-1649.DOC
061804

(X) Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Nancy W. Vensko
Registration No. 36,298
Attorney of Record
Customer No. 20,995
(805) 547-5580

LAMEND-TRANS
O:\DOCS\NWV\NWV-1651.DOC:vb
062104

The interaction of FPRL1 with serum amyloid A (SAA)